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Research Article

ACUTE ORAL TOXICITY OF *SINAPIS ALBA* IN SWISS ALBINO MICE

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ABSTRACT

The objective of this study to investigate the acute oral toxicity effects of *Sinapis alba* seeds in swiss albino female mice based on OECD guideline 423. The ethanolic extract was administered orally at two different doses of 300 mg/kg and 2000 mg/kg of body weight. The female mice were observed continuously for 4 hrs for behavioural and autonomic profiles and for any other sign of toxicity or mortality up to a period of 14 days. In between 14 days observation; body weight, feed, and water intake were also observed on 1st, 7th and 14th day. At the end of 14 day's observation, female mice from each group were sacrificed by cervical dislocation and vital organs were removed, weighed and made histological sections. All results were shown that there were no any toxic effects, feed and water intake were maximum on 7th day. Body weight, organ weight and histological sections were also normal.

Keywords: Acute oral toxicity study, *Sinapis alba* seeds ethanolic extract, Feed and water intake, Body weight, Vital organ weight, Histology.

INTRODUCTION

Sinapis alba (Brassicaceae) commonly known as white mustard. Varieties of mustard are a condiment that has been used for culinary, religious and cultural purposes by humanity since time immemorial. Mustard has figured prominently in the Indian tradition and its medicinal properties have been systematically evaluated and documented in the classical ayurvedic texts. *S alba* is native to the mediterranean region. It grows on fields and waste areas, on calcareous soils [1]. White mustard is an annual plant, with an erect steam and numerous branches spring from the main stem. Leaves are alternately arranged, etiolate and serrated, with short, white bristles along the veins. Flowers are pale yellow, forming a shape of a cross. Flowering occurs from June to August. Seeds are globular, dark reddish-brown [2-6].

White mustard seeds of *S. alba* contain a bland fixed oil 23-25%, a crystalline substance called "sinalbin," sinapinsulphocyanide, lecithin, mucilage (only in testa); myrosin a ferment; proteins, ash 4%, consisting of the phosphates of potassium, magnesium, and calcium. Proteins, water, vitamin-A, thiamine (B_1), riboflavine [7]. Taste of *S. alba* is pungent, bitter, unctuous, sharp and hot.

Mustard flour of *S. alba* is nervine stimulant, emetic and diuretic. In large doses, it is stimulant, emetic and narcotic-poison when given with hot water. Volatile oil is a stimulant, rubefacient and vesicant [7]. In the ayurvedic system of medicine, different preparations of *S. alba* are recommended for epigastrium in obstinate vomiting, cholera, spasmodic whooping cough, delirium, apoplexy, hysteria, swollen joints, epilepsy, migraine, cough, cold, flue and its oil works as an appetizer [7,8]. Ayurveda also explains that white mustard pacifies kapha and vata and is useful for diseases of ear and head, and it has antiepileptic, antimicrobial activity and increases digestive power and is useful in management of iching, skin diseases and intestinal worms [6,9]. It can also used for purging the body of toxins. Mustard seed is an ingredient of formulations that induce emesis, cleanse the cranial cavity and for giving decoction enema and these procedures are indicated in diseases like vomiting, insanity, flatulence, pallor, jaundice and rhinitis.

Apart from the scientific study of various activites of *S. alba*, there is very less scientific data of toxicity study available. Use of plants as a source of medicines has been inherited and is an important component of the health care system in India. In the Indian systems of medicine, most practitioners formulate and dispense their own recipes; hence this requires proper documentation and research. In the western world also, the use of herbal medicines is steadily growing with approximately 40% of population reporting use of herb to treat medical illnesses within the past year [10,11]. Therefore, it is necessary to study whether *S. alba* has any toxic effects or not if use and this study also help in selection, preparation of the appropriate dose of drugs. Hence, the present study was undertaken to evaluate the acute oral toxicity study of *S. alba* as per OECD guideline-423.

MATERIALS AND METHODS

Plant materials and preparation of drug solution

The ethanolic extract of *S. alba* was used for acute oral toxicity study. Stock solution was freshly prepared in distilled water before dosing from which the 300 mg/kg and 2000 mg/kg doses were administered by selecting the appropriate concentration. Dosing was done only on 1st day of 14 days of observation.

Experimental animals and housing conditions

Healthy female swiss albino mice (20-25 g) were used. They were maintained at 25±2°C, relative humidity of 45-55% and under standard environmental conditions with 12:12 hrs light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Nutrivet life sciences, Pune) and water was given *ad libitum*. The Institutional Animal Ethics Committee approved the protocol (257/CPCSEA). All the experiments were carried out between 9:00 hrs and 16:00 hrs.

Experimental design

A total of 18 female swiss abino mice (6 mice/group), were randomly selected and marked for individual identification. All the animals were subjected to 4 hrs of fasting prior to treatment. The test groups included a control group (10 ml/kg distilled water) and two other treatment groups *viz.* Group I and Group II for dose 300 mg/kg and 2000 mg/kg body weight of extract, respectively.

The animals were observed for 1 hr after treatment, and then intermittently for 4 hrs, and thereafter the mice were further observed for up to 14 days following treatment. Clinical signs such as weakness or aggressiveness, food refusal, loss of weight, diarrhea, discharge from eyes and ears, noisy breathing, and the number of deaths in each

treated group were monitored carefully. Body weight, food and water intake were measured on 1st, 7th and 14th day. Subsequently, the animals were sacrificed by cervical dislocation. Vital organs including the heart, kidney, liver, lung, and spleen were removed for macroscopic analysis, and weighed and preserved in 10% buffered formalin solution [12,13] and processed routinely and stained with hematoxylin and eosin. The sections were examined with image microscope (Magnus, Pune) under ×4, ×10, ×40 magnification values for identification of any lesions, degeneration, necrosis, hemorrhage.

Statistical analysis

The result of body weight, feed and water intake and organ weight were expressed as mean±standard error mean (n=6). The data were analyzed by using one-way analysis of variance, followed by Dunnett's multiple comparison test. Significance set at *p<0.05, **p<0.01.

RESULT

There were no evidence of depression, diarrhea, dizziness, salivation, tremors, coma or any behavioral changes and no mortality found in animals even after 14 days. All of the control and treated mice survived until the end of the treatment period.

During 14 days of observation, body weight was measured on 1st, 7th and 14th day of observation days and result showed that there was no significant change in weight gain or weight loss (Table 1). However, there were changes in food and water intake. Till 7th day of observation days, food and water intake were significantly increased (p<0.01) while on 14th day of observation, there were no any significant increase in food and water intake as compared with control group (Tables 2 and 3).

Table 1: Body weight (g) of female swiss albino mice of each group

Day	Control (10 ml/kg)	Group I (300 mg/kg)	Group II (2000 mg/kg)
On 1st day	36.29±1.48	41.25±1.29	34.11±0.97
On 7th day	36.78±1.43	42.65±1.21	36.38±1.08
On 14th day	36.98±1.27	41.9±1.76	35.9±1.46

Values are expressed as mean±SEM (n=6), n-number of animals, *p<0.05, **p<0.01 compared with vehicle-treated control female swiss albino mice, (ANOVA followed by Dunnett's test), SEM: Standard error of mean

Table 2: Food intake (g) of female swiss albino mice of each group

Day	Control (10 ml/kg)	Group I (300 mg/kg)	Group II (2000 mg/kg)
On 1st day	3.98±0.17	4.88±0.15	4.42±0.17
On 7th day	4.33±0.10	5.24±0.17	5.65±0.19**
On 14th day	4.47±0.15	5.31±0.21	4.76±0.13

Values are expressed as mean±SEM (n=6), n-number of animals, *p<0.05, **p<0.01 compared with vehicle-treated control female swiss albino mice, (ANOVA followed by Dunnett's test), SEM: Standard error of mean

Table 3: Water intake (ml) of female swiss albino mice of each group

Day	Control (10 ml/kg)	Group I (300 mg/kg)	Group II (2000 mg/kg)
On 1st day	4.28±0.25	5.38±0.17	4.51±0.18
On 7th day	4.30±0.20	5.64±0.19	8.10±0.27**
On 14th day	4.97±0.17	5.54±0.23	4.66±0.13

Values are expressed as mean±SEM (n=6), n-number of animals, *p<0.05, **p<0.01 compared with vehicle-treated control female swiss albino mice, (ANOVA followed by Dunnett's test), SEM: Standard error of mean

After completion of 14 days of observation, vital organs were removed and measured the relative organ weight. The results showed that there was no significant change in organ weight gain or loss (Table 4).

After histology study, staining of all vital organs like heart, liver, lungs, kidney and spleen showed normal histoarchitecture (Figs. 1-5).

The microscopic evaluations of the selected organs did not reveal any abnormalities like lesions, degeneration, necrosis, hemorrhage or inflammatory exudates that could be attributed to the oral administration of *S. alba* extract to the female mice.

Table 4: Organ weight (mg) of female swiss albino mice of each group

Organs	Control (10 ml/kg)	Group I (300 mg/kg)	Group II (2000 mg/kg)
Heart	179.23±6.19	203.06±8.55	173±7.08
Kidney	1878.36±64.94	2128.07±89.62	1823.34±74.20
Liver	494.78±17.17	560.56±23.60	480.29±19.54
Lung	270.41±9.33	306.62±12.91	262.71±10.69
Spleen	207.15±7.16	234.69±9.88	201.09±8.18

Values are expressed as mean±SEM (n=6), n-number of animals, *p<0.05, **p<0.01 compared with vehicle-treated Control female swiss albino mice, (ANOVA followed by Dunnett's test), SEM: Standard error of mean

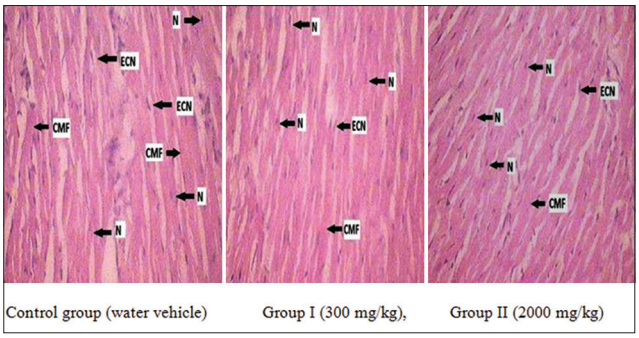

Fig. 1: Heart of female swiss albino mice of each group, CMF: Cardiac muscle fibre, ECN: Endothelial cell nucleus, N: Nuclei

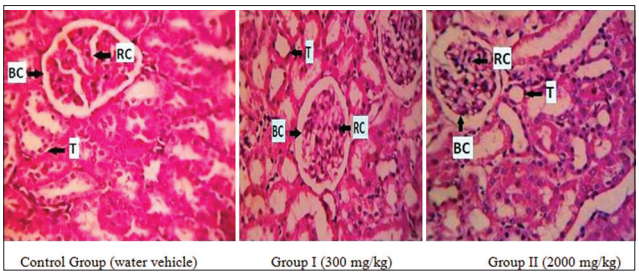

Fig. 2: Kideny of female swiss albino mice of each group, RC: Renal capsule, BC: Bowman's capsule, T: Tubule

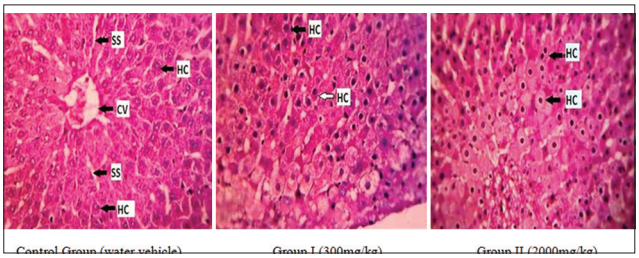

Fig. 3: Liver of female swiss albino mice of each group, CV: Central vein, SS-Sinusoidal Dilation, HC: Hepatic cell

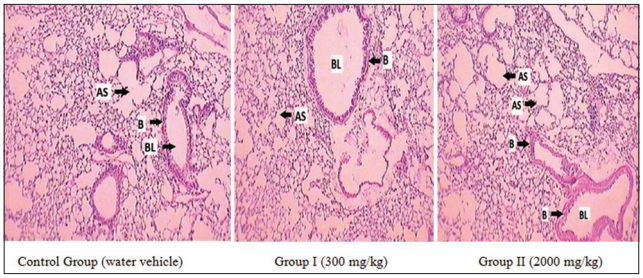

Fig. 4: Lung of female swiss albino mice of each group, AS: Alveolar air space, B-Bronchioles, BL: Bronchiolar lumen

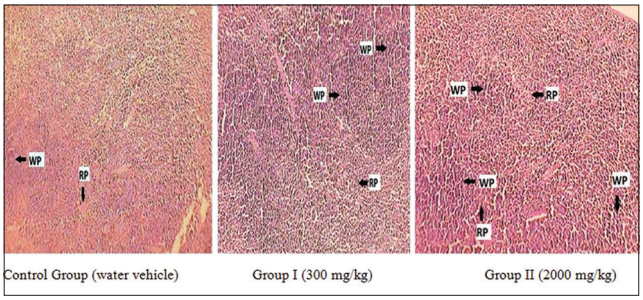

Fig. 5: Spleen of female swiss albino mice of each group, RP: Red pulp, WP: White pulp

DISCUSSION

Herbal medicines have been used world-wide for thousands of years. These herbs mainly originate from plants, minerals and animal products and may be used either in their primary, minerals and animal products and may be used either in their primary forms or combined into mixtures. Herbal preparations can also be formulated into tablets, pills and liquids, as well as being commercially available in the form of proprietary medicines. The purpose of medicinal use of herbs may vary with the different traditional medicinal system of different societies, but can simply be considered as being either for the promotion of health or the relief of ailments [14-16].

Traditionally, there is a myth that herbs have been considered to be non-toxic and even harmless, mainly because of their natural origin. A World Health Organization (WHO) survey indicated that about 70-80% of the world's population rely on non-conventional medicine, mainly of herbal source in their primary healthcare [17-19].

Although medicinal plants may produce several biological activities in humans, generally very less data is available about their toxicities [10,20] and this lack of information makes it difficult to compare the benefit-risk ratio profile of herbal medicines. However, both adverse drug reactions and poisonings associated with the use of herbal medicines have increasingly been reported last few years [10,16].

In daily practice, many herbal poisonings were not diagnosed or treated correctly and therefore, more information about herbal toxicology is urgently needed. Though, the medicinal plants are considered to be non-toxic, the sustenance of life, can be toxic, if consumed too much or in an inappropriate proportion. Thus, the key aspect to understanding the toxicity of materials is to know how much of a substance dose can cause harm regarding to safety and efficacy. Hence, the scientific approach through experimental and clinical validation of efficacy and documentation of useful herbs, herbal preparations and other formulations is necessary, is done in modern medicine, animal toxicity studies are also required to establish the potential adverse effects [21].

In this acute oral toxicity study of *S. alba,* there were no any toxic signs or mortality even after 14 days of observation and also there were no any significant changes in body weight, organ weight, but on 7th day of observation, there were significant increase in food and water intake. These results showed there may be no toxic effect in acute oral toxicity, but this herb may increase apetite [8] till 7 days even after only 1st day of dosing. In histology study of all groups of vital organs such as heart, kidney, liver, lungs and spleen showed normal histoarchitecture and there were no any lesion, necrosis, hemorrhage, degeneration. That is, there was no any toxicants accumulation in organs.

CONCLUSION

This study shows that ethanolic extract of *S. alba* does not have any toxic effects in acute oral toxicity study but it has strong appetizer activity. Sub and chronic toxicity, mutagenicity and carcinogenicity studies are essential to further support the safe use of these plants.

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